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Seasonal fluctuation of *Plasmodium falciparum* gametocytaemia

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Abstract

Two numerically minor components of *Plasmodium falciparum* prevalence—gametocytaemia and trophozoite densities >99/500 white blood cells—displayed an annual cycle that reflected the seasonal abundance of infective *Anopheles dirus* at a hyperendemic focus in Thailand, even though the gross monthly prevalence for combined ages remained stable. Gametocyte prevalence rose more than 300% within 30 d after the capture of the dry season's first infective mosquito, remained at about 8% until the beginning of the monsoon 7 months later, then fell within 60 d to about 2%. The number of cases with a high density of trophozoites behaved similarly. These periodic fluctuations represented changes in incidence, at least half of which appeared to be due to superinfection. Almost 49% of all gametocyte carriers were older than 14 years, but nearly all gametocyte densities >20/500 white blood cells were in children. These observations, as well as the calculated efficiency of human infectivity, imply that superinfection of adults may contribute significantly to transmission in semi-immune populations.

Introduction

Plasmodium falciparum is usually first detected in the peripheral circulation of immunologically naive humans as trophozoites 7–11 d after a successful sporozoite inoculation. The number of parasites multiplies exponentially at each 48 h schizogonic cycle, reaching its highest density 5–8 d after initial patency. After this asexual peak has subsided, or about 3 weeks after inoculation, gametocytes, which until then have been absent, suddenly flood the circulation. Large numbers of these persist for about 2 weeks, but thereafter they are relatively rare and show no marked periodicity (GARNHAM, 1966; JEFFERY *et al.*, 1959). Since gametocytes are the sexual stage of malaria and propagate only in the mosquito, transmission of the disease is a direct consequence of their availability.

Gametocytes tend to be disproportionately scarce during inter-epidemic periods (SCHUFFNER, 1938; MACDONALD & MAJID, 1931) but particularly infective during active transmission (RUTLEDGE *et al.*, 1969). At a hyperendemic focus in East Africa, WILSON (1936) observed that a sudden increase in numbers of infective mosquitoes was followed by an increase in gametocyte prevalence, even though *P. falciparum* trophozoite prevalence remained stable. He interpreted the rising number of gametocyte carriers as indirectly representing new infections; because his mosquito and blood collections were not

always made at the same times, he was unable to say precisely what the relationship was. More recently a seasonal increase in parasite density and prevalence of gametocyte carriers has been noted in northern Nigeria (MOLINEAUX & GRAMICCIA, 1980) and a seasonal increase in morbidity in The Gambia (GREENWOOD *et al.*, 1987); at both places there were also relatively slight fluctuations in gross prevalence. An increase in numbers of cases without a matching increase in prevalence is a paradox most easily resolved by assuming that substantial superinfection took place.

Superinfection is an important component of malaria epidemiology, but one which is difficult to quantify. Naturally acquired immunity to malaria greatly mitigates the risk of being ill from the disease, but does not prevent reinfection, which may occur before parasites from a previous infection have disappeared. The greater the ratio of incidence to rate of recovery, the greater the chance of superinfection; in some hyperendemic communities many children seem to be continuously infected. Above a threshold determined by variables specific to the situation, increasing incidence no longer increases prevalence, yet there is evidence that it does affect rates of mortality, morbidity, splenomegaly, and parasite density (MACDONALD, 1951). We have conjectured that differences in disease patterns between some Asian and African examples of hyperendemicity are due to differences in inoculation rates and size of inocula (ROSENBERG *et al.*, 1990a).

A hyperendemic community in Thailand had a gross monthly prevalence of *P. falciparum* infection that was essentially stable (ROSENBERG *et al.*, 1990b), even though the abundance of infective *Anopheles dirus*, the predominant vector, was seasonal (ROSENBERG *et al.*, 1990a). In this report we show how seasonal oscillations of gametocytaemia and high trophozoite densities reflected incidence, some of which may have been due to superinfection, and how the timing of these oscillations was related to changes in vectorial capacity. We discuss how increased numbers of gametocyte carriers at the time when vector survival was longest may have influenced transmission.

Methods

Site

The study area, part of Ban Phluang village, was in south-eastern Thailand. The inhabitants (approximately 250) were relatively prosperous cultivators of rubber, fruit trees and rice, and seldom left the settlement. Houses were in or near orchard and scrub, and intense malaria transmission occurred in the village itself, despite the presence in most houses of

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mosquito nets. Almost all malaria was transmitted during the November–May dry season. About half those older than 12 years spent at least one night per week during the dry season tapping rubber near the village; tapping was done between midnight and dawn, when vector activity was high (ROSENBERG *et al.*, 1990a). A more detailed description of the topography, demography, and economy of the area is given by ROSENBERG *et al.* (1990b).

Blood collection and examination

Thick and thin blood films were collected for 24 consecutive months from nearly all inhabitants; data were analysed from those contributing at least 8 specimens per year, 176 people in the first year and 186 in the second. Thick films were stained with Giemsa's stain and the volume containing 500 white blood cells (WBC) was examined at a magnification of 1000 \times , a procedure requiring about 10–15 min per slide by a single microscopist; there were approximately 5500 WBC/mm³ of a stained thick blood film (ROSENBERG *et al.*, 1990b). Thick films made in April 1984 were accidentally fixed and no gametocyte rate or parasite densities are available for that month. The 36% of cases with the highest densities—which contained more than 76% of all densities >99/500 WBC—were treated monthly. *P. vivax* was treated with chloroquine and primaquine; *P. falciparum* was

treated in the first year with quinine, tetracycline and primaquine and in the second year with mefloquine+sulfadoxine+pyrimethamine and primaquine. Mefloquine does not affect the viability of gametocytes present at the time of treatment or sporogonic development from them (HARINASUTRA *et al.*, 1987); nor do sulphonamides and pyrimethamine seem to have any gametocidal or sporontocidal activity at concentrations typically found in humans treated for malaria (SCHOLER *et al.*, 1984). Details of examination and treatment are given in a previous report (ROSENBERG *et al.*, 1989b).

Mosquito infection

Man-biting *Anopheles* were collected outdoors from 1900 h to 0450 h for 7 consecutive nights per month for 2 years by 2-man teams working simultaneously at each of 2 unchanging sites within the village (ROSENBERG *et al.*, 1990a). Collections began the night following each month's blood collection. An extraordinary collection was made between 2 and 7 November 1983. After species identification, *Anopheles* were dissected to determine parity and midgut and salivary gland infection rates. The species of sporozoites found was determined using indirect fluorescent antibody or enzyme-linked immunosorbent assays. In this report only mosquito infectivity (the presence of sporozoites in glands) is considered, and no distinction is made between sites or hours of capture.

Results

In the first year 5.3% of *P. falciparum* infections included, or were composed solely of, gametocytes; in the second year, the figure was 4.2%. Most gametocyte cases (88.3%) also had trophozoites. In the first year 96 cases with gametocytaemia occurred in 65 people (36.9% of the study population), 18 of whom accounted for 51.0% of the cases by being positive more than once; in the second year, 83 cases occurred in 65 people, 11 of whom accounted for 34.9% of the cases. Of gametocytaemias found in the same individuals, only 12 cases in the first year and 8 in the second occurred consecutive to each other, suggesting that many represented fresh rather than recurrent infections. Gametocyte densities ranged from 1 to 2952 per 500 WBC, or about 12–35424 per mm³; the geometric mean for all ages was 3.8/500 WBC, or about 46/mm³.

The number of people showing *P. falciparum* gametocytes in their blood increased more than 300% each December (Figure, A) or within 30 d after catching the first infective mosquito of the transmission season (Figure, C). The number of gametocyte carriers remained high throughout the dry months, November to May, but then steeply declined soon

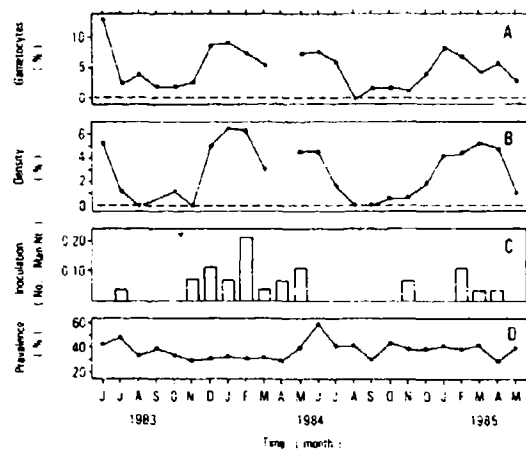


Figure. Monthly relationship between (A) prevalence of *Plasmodium falciparum* gametocytes, (B) prevalence of *P. falciparum* trophozoite densities >99/500 white blood cells, and (C) inoculation rate by *Anopheles dirus* (infected bites per man-night); gross *P. falciparum* prevalences (D) are shown for comparison. April 1984 data for A and B are missing; inoculation rate during extraordinary November, 1983 collection is represented by asterisk (*).

Table 1. Seasonal distribution by age of *Plasmodium falciparum* gametocyte carriers

Age group*	Wet season (June–October)			Dry season (November–May)		
	Year 1	Year 2	Total	Year 1	Year 2	Total
≤14 years	5.7% (263) ^b	0.9% (216)	3.6% (479)	12.0% (366)	6.7% (432)	9.2% (798)
>14 years	0.8% (509)	1.3% (462)	1.0% (971)	5.3% (617)	10.0% (907)	8.1% (1524)
Total	2.5% (772)	1.2% (678)	1.9% (1450)	7.8% (983)	9.0% (1339)	8.9% (2322)

*All comparisons within age groups are significant (*t* test, $P < 0.05$), except for age group >14 years in wet season; all comparisons between age groups are significant (*t* test, $P < 0.05$), except for totals in dry season.

^bNumbers in parentheses are numbers of specimens examined including negative slides.

after the last infective mosquito was found, about the time the annual monsoon began (Figure, A). A disproportionately high number of gametocyte carriers ($G=7.4$, $P<0.01$; SOKAL & ROHLF, 1981) was found between November and May (Table 1); gametocyte densities were also higher then.

Persons aged more than 14 years comprised 67% of the population and harboured 49% of the gametocytaemias; but 84.6% of gametocyte densities $>20/500$ WBC were in those aged 14 years or less. Dry season prevalence was 2.6 times higher than wet season prevalence in children, compared to 8.0 times higher in those over 14 years old, a relationship that has also been found in Nigeria (MOLINEAUX & GRAMICCIA, 1980), and which may reflect more conscientious treatment of children. The second year the inoculation rate fell to about one-third of that in the first year (ROSENBERG *et al.*, 1990a), and the percentage of the population aged 14 years or less carrying gametocytes decreased significantly ($G=4.53$, $P<0.05$), but the proportion of those over 14 years who were carriers increased (Table 1). This suggests that those over 14 years of age were also acquiring infections at some place where the risk was greater than in their homes.

Table 2. Observed prevalences of infected humans compared with theoretical prevalences calculated from parous rates and their 95% confidence limits*

	Year 1 ^b	Year 2 ^c
Parous (%)	0.70 (0.65-0.74) ^d	0.58 (0.53-0.63)
Daily survival (p)	0.89 (0.87-0.91)	0.83 (0.81-0.86)
Vectorial capacity (C)	1.28 (0.79-1.92)	0.48 (0.29-0.79)
Infective prevalence (y)	0.078 (0.13-0.05)	0.063 (0.10-0.04)
Observed gametocyte prevalence	0.076	0.065

**Plasmodium falciparum* and *P. vivax* combined; p =parous^{1,3}, $C=(ma)ap^b/\ln p$; $y=h^c/C$.

^bNovember-May, 1983; man-biting rate (ma)=1.93; inoculation rate (h^c)=0.10; assumed biting frequency (a)=0.33; days between blood meals (n)=3.

^cDecember-May, 1984: man-biting rate (ma)=2.33; inoculation rate (h^c)=0.03; assumed biting frequency (a)=0.33; days between blood meals (n)=3.

^dNumbers in parentheses are ± 2 standard errors for the parous rates, or are calculated from these two values for the other quantities.

If it is assumed that vectorial capacity (C) (GARRET-JONES & SHIDRAWI, 1969) is unchanging, then the prevalence of infective mosquitoes (y) needed to account for a given inoculation rate (h^c) can be estimated from $y=h^c/C$ (DIETZ *et al.*, 1974). This has been done in Table 2 for both dry seasons using C calculated from the mean observed parous rate of *An. dirus* (ROSENBERG *et al.*, 1990a) and from its 95% confidence limits (MEILLON *et al.*, 1967). A comparison of the observed gametocyte prevalences of 0.076 in the first year and 0.065 in the second with the range of calculated human infectiousness (Table 2) suggests a high level of gametocyte infectivity. If daily mosquito survival is assumed to have been at the upper end of the confidence limit—only slightly greater than that calculated from mean parity—and the infectiousness of people with negative blood films is disregarded, then infectivity was nearly 68% in the first year and 60% in the second. Lower survival rates would require higher, and increasingly unlikely, efficiency of transmission (COVELL, 1960).

The prevalence of *P. falciparum* trophozoite densities higher than 99/500 WBC (about 1200/mm³) showed a seasonal pattern similar to that of gametocytaemia: only 5.8% of high densities occurred during the rainy season (Figure, B). Of the 103 densities $>99/500$ WBC, 36.1% were in people not patent for *P. falciparum* during the preceding month and 14.6% followed parasitaemias also $>99/500$ WBC, suggesting that at least the remaining half of the high densities resulted from superinfection. For all ages combined, densities $<100/500$ WBC comprised 93% of all *P. falciparum* infections (ROSENBERG *et al.*, 1990b) and did not have a seasonal pattern, although they appeared somewhat more variable during the wet season (Figure, D). Children below 10 years of age, however, had monthly *P. falciparum* trophozoite prevalences significantly higher than those of adults during 3 sustained periods: June–August 1983, February–March 1984, and February–April, 1985 (Table 3). The inoculation rates before June 1983 are unknown, but February was a month of high transmission in both years (Figure, C).

No *P. vivax* periodicity was apparent in either gametocytaemia or high densities at any age. Over 24 months, 48 infections with *P. vivax* gametocytes

Table 3. Seasonal fluctuation of prevalence of *Plasmodium falciparum* asexual forms by age

Month	Year 1 (1983–1984)			Year 2 (1984–1985)		
	<10 years	≥ 10 years	Difference ^a	<10 years	≥ 10 years	Difference ^a
Jun.	57.6	37.3	20.3 ^a	63.6	60.5	3.1
Jul.	67.6	41.9	25.7 ^b	48.6	38.9	9.7
Aug.	45.9	30.8	15.1	50.0	40.8	9.2
Sep.	45.9	36.4	9.5	44.1	27.2	16.9
Oct.	43.2	31.6	11.6	48.6	43.4	5.2
Nov.	27.8	30.0	-2.2	43.8	38.4	5.4
Dec.	36.1	29.8	6.3	43.6	54.7	8.9
Jan.	27.5	35.1	-7.6	40.0	40.6	-0.6
Feb.	57.1	24.4	32.7 ^b	60.0	33.1	26.9 ^b
Mar.	44.1	27.1	17.0 ^b	56.1	38.2	17.9 ^b
Apr.	—	—	—	40.0	25.0	15.0
May	48.4	37.9	10.5	41.0	38.2	2.8

^aAdult prevalence subtracted from child prevalence.

^bDifference greater than expected (χ^2 test, $P<0.05$).

occurred among 40 people. Gross *P. vivax* prevalence dropped from 12.8% in the first year to 7.2% in the second; 93.8% of blood films containing gametocytes also included trophozoites. Gametocyte densities ranged from 1 to 75/500 WBC (median=2.5).

Discussion

The perennial, monsoon-generated cycle of malaria incidence at Ban Phluang was more evident in the seasonal fluctuation of high trophozoite densities and of gametocyte prevalence than in the commonly used index of gross parasite prevalence. We observed a temporal relationship between transmission, high densities, and gametocytaemia that was consistent with the known biology of *P. falciparum*. WILSON (1936) interpreted his African data as showing that gametocyte prevalence peaked after transmission had stopped, leading him to conclude that the 2 events were only indirectly related; he may have been misled by inconsistencies in his study design, as his collection of mosquitoes and blood data were neither synchronized nor always regular, making precise alignment of cause and effect difficult. Nonetheless, the pattern we observed in Thailand was strikingly similar to that which WILSON (1936) described for Tanzania 50 years earlier; the similarity is all the more remarkable as the 2 sites have virtually no features in common other than seasonally intense transmission.

Except in infants, reinfection and superinfection accounted for all malaria incidence at Ban Phluang. Differentiating between reinfection and superinfection is problematical when low densities make the identification of cases uncertain (EARLE *et al.*, 1939; MILLER, 1958), but it appears that at least half of the densities >99/500 WBC were superimposed on current, low-level infections. One effect of superinfection should be to decrease the recovery rate (ARON & MAY, 1980), explaining why prevalence remains high during brief periods of low transmission. Superinfection also explains why at least some incidence was manifested by a rise in the numbers of gametocyte carriers but not in trophozoite prevalence. It is less clear why reinfections, which may have accounted for half of the incidence, did not inflate prevalence soon after transmission recommenced. Although annual *P. falciparum* incidence was at least 100% (ROSENBERG *et al.*, 1990b), fewer than one-third of the population were ever found to have an elevated trophozoite density or a gametocytaemia, and it is possible that many reinfections may have been initially subpatent.

The influence of superinfection on the chronicity of hyperendemic malaria has been an important component in attempts to understand the epidemiology of the disease (BAILEY, 1982). It should also be considered in quantifying transmission if, as seems evident from our data, it alters the infectiousness of man to vector. But the most ambitious transmission model yet tested is based in part on the explicit assumption that 'superinfection prolongs parasitaemia, without effect on infectivity' (DIETZ *et al.*, 1974). Although seasonal incidence at Ban Phluang disproportionately increased the prevalence of gametocytaemia in adults, any increase in gametocyte prevalence, even at the low densities typical of semi-immunes, almost certainly favoured parasite dissemination.

The contribution to transmission made by low density infections in adults has been controversial. It

was once presumed to be insignificant (MACDONALD, 1951), but it has been proved that even sub-patent infections can infect mosquitoes (COVELL, 1960). Except for the 13% of the population aged below 5 years, gametocyte densities at Ban Phluang were the same in all age groups (ROSENBERG *et al.*, 1989b) and it seems probable that, as in Africa (MUIRHEAD-THOMSON, 1957), the proportionately large number of semi-immunes played a crucial part in transmission. The actual efficiency of human infectivity was unlikely to have been as high as we calculated using the Dietz-Molineaux-Thomas model (Table 2); considering the large number of approximations used and the relatively small sample sizes, it may be that the validity of the equation cannot be evaluated without feeding uninfected mosquitoes on a representative sample of the population.

Each year transmission began during the last weeks of the annual monsoon as unknown factors, probably micro-climatic, suddenly began to favour prolonged survival of the vector (ROSENBERG *et al.*, 1990a). For a period of less than 30 d high survival rates coincided with high adult emergence, causing an explosive increase in vectorial capacity. At that initial point gametocyte prevalence was low, but so presumably was immunity to reinfection. The great rapidity with which gametocytes and high densities ascended to something of a plateau suggests that incidence also rapidly rose and levelled. This implies that the basic reproductive rate was high and that the susceptible population was sufficiently large to provide the critical size necessary to maintain a stable prevalence (ARON & MAY, 1980). To what extent increased gametocyto-genesis enhanced transmission after mosquito survival improved is unclear, but the large drop in vectorial capacity after the initial increase (ROSENBERG *et al.*, 1990a) did not lower gametocyte prevalence, suggesting that compensation occurred.

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